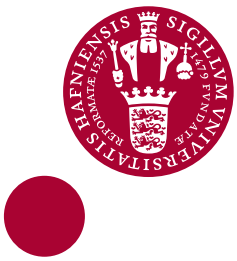

DHA and improvement of memory function: evaluation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006

(Scientific Opinion)

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)

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EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)

Abstract

Following an application from DSM Nutritional Products, submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of the United Kingdom, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to docosahexaenoic acid (DHA) and improvement of memory function. DHA is sufficiently characterised. An improvement of memory function is a beneficial physiological effect. In weighing the evidence, the Panel took into account that, out of the 11 human intervention studies from which conclusions can be drawn for the scientific substantiation of the claim, two studies showed a beneficial effect of DHA supplementation on memory function, one study showed inconsistent results, one study showed a negative effect of DHA on memory function and seven studies did not show an effect of DHA on memory outcomes. The Panel considers that the majority of the human intervention studies provided did not show an effect of DHA supplementation on memory, and that the conflicting results across studies cannot be explained by differences in the study design, the source of DHA, the DHA dose, the baseline characteristics of the subjects recruited, or the duration of the studies. The Panel also took into account that the meta-analyses of intervention studies submitted by the applicant do not provide evidence for an effect of DHA on memory function, and that the three observational studies from which conclusions could be drawn for the scientific substantiation of the claim do not provide evidence for an association between dietary DHA and memory function. The Panel concludes that a cause and effect relationship has not been established between the consumption of DHA and an improvement of memory function.

Keywords: DHA, fish oil, memory, cognitive function, health claims

Requestor: Competent Authority of the United Kingdom following an application by DSM Nutritional Products.

Question number: EFSA-Q-2015-00456

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Summary

Following an application from DSM Nutritional Products, submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of the United Kingdom, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to docosahexaenoic acid (DHA) and improvement of memory function.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.

The food constituent that is the subject of the health claim is DHA (22:6 n-3) which is a well-characterised n-3 long-chain polyunsaturated fatty acid. The Panel considers that DHA is sufficiently characterised.

The claimed effect proposed by the applicant is 'contributes to improved memory function'. The target population proposed by the applicant is 'the general healthy adult population'. Memory is the cognitive ability to maintain previously learned information, so that it may be accessed and used at a later time. Memory is not a unitary construct but instead reflects a number of distinct cognitive processes (e.g. episodic memory, working memory, short-term memory, semantic memory). The Panel considers that an improvement of memory function is a beneficial physiological effect.

The applicant identified 15 human intervention studies and 13 observational studies as being pertinent to the claim. The applicant also provided three meta-analyses using data from the 15 intervention studies.

No conclusions could be drawn from four out of the 15 human intervention studies owing to methodological limitations. Out of the 11 intervention studies from which conclusions could be drawn for the scientific substantiation of the claim, two were performed with DHA from microalgae, eight with DHA from fish oil and one with phosphatidylserine (PS)-DHA. One study conducted with DHA from algal oil showed an improvement in episodic memory but not in working memory in healthy older adults with age-related cognitive decline after consumption of 900 mg DHA for 24 weeks, while the other study with DHA from the same source showed a negative effect on episodic memory and no effect on semantic memory in young adults after consumption of 400 mg DHA for 50 days. Out of the eight intervention studies performed with DHA from fish oil, which provided daily doses of DHA ranging from 252 mg to 1.55 g for periods between 12 weeks and 24 months, only one showed an improvement in memory in adults with mild cognitive impairment, while the results from another study were inconsistent and six studies did not show an effect of DHA supplementation on memory outcomes. The study which used PS-DHA did not show an effect of the food on memory function.

The applicant conducted meta-analyses using data from the 15 intervention studies. The overall between-group models, which included all dose levels of DHA, all sources of DHA, and all subjects regardless of their age or cognitive status at baseline, showed no effect of DHA supplementation on episodic, semantic or working memory. The Panel considers that these meta-analyses do not provide evidence for an effect of DHA on memory function.

The applicant provided 13 observational studies as being pertinent to the claim. Among the references provided, nine studies did not report on dietary intakes of DHA but addressed the association between blood concentrations of DHA and measures of memory. One study reported only a composite cognitive score but no independent memory test scores. No conclusions can be drawn from these 10 studies for the scientific substantiation of the claim. The three remaining observational studies from which conclusions can be drawn for the scientific substantiation of the claim did not provide evidence for an association between dietary DHA and memory function.

In weighing the evidence, the Panel took into account that, out of the 11 human intervention studies from which conclusions can be drawn for the scientific substantiation of the claim, two studies showed a beneficial effect of DHA supplementation on memory function, one study showed inconsistent results, one study showed a negative effect of DHA on memory function and seven studies did not show an effect of DHA on memory outcomes. The Panel considers that the majority of the human intervention studies provided did not show an effect of DHA supplementation on memory, and that the conflicting results across studies cannot be explained by differences in the study design, the source of DHA, the DHA dose, the baseline characteristics of the subjects recruited or the duration of the studies. The Panel also took into account that the meta-analyses of intervention studies submitted by the applicant do not provide evidence for an effect of DHA on

memory function, and that the three observational studies from which conclusions could be drawn for the scientific substantiation of the claim do not provide evidence for an association between dietary DHA and memory function.

The Panel concludes that a cause and effect relationship has not been established between the consumption of DHA and an improvement of memory function.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1924/2006[1] harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children's development and health) which are based on newly developed scientific evidence, or which include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of this Regulation, an application for inclusion in the Community list of permitted claims referred to in Article 13(3) shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

1.2. Interpretation of the Terms of Reference

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: docosahexaenoic acid (DHA) and improvement of memory function.

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of DHA, a positive assessment of its safety, nor a decision on whether DHA is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

1.3. Additional information

Claims on DHA and maintenance of normal brain function (EFSA NDA Panel, 2010) and contribution to normal brain development (EFSA NDA Panel, 2014) have already been assessed by the Panel with favourable outcomes.

2. Data and methodologies

2.1. Data

2.1.1. Information provided by the applicant

Food/constituent as stated by the applicant:

- According to the applicant, the food which is the subject of the health claim is DHA.

Health relationship as claimed by the applicant:

- According to the applicant, DHA contributes to improved memory function.
- The applicant states that this improvement of memory function could be assessed by improvements in tests of episodic memory following DHA supplementation from a variety of sources.
- With regard to a possible mechanism, the applicant claims that DHA has been shown to enhance neurotransmission via increased synaptic plasticity of the glutamatergic synapses in the hippocampus and pre-frontal cortex, to protect the glutamatergic synapses via regulation

[1] Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

of astrocyte function and plasticity, and to support adult neurogenesis by maintaining active proliferative pools and favouring neuronal maturation.

Wording of the health claim as proposed by the applicant:

- 'DHA contributes to improved memory function'.

Specific conditions of use as proposed by the applicant:

- The applicant has proposed an intake of ≥ 580 mg DHA/day. According to the applicant, this level of daily intake could be obtained from conventional foods, fortified foods, food supplements or a combination of these.
- The target population proposed by the applicant is the general healthy adult population.

2.1.2. Data provided by the applicant

Health claim application on DHA, alone or in combination with eicosapentaenoic acid (EPA), contributes to improved memory function pursuant to Article 13.5 of Regulation 1924/2006, presented in a common and structured format as outlined in the Scientific and technical guidance for the preparation and presentation of applications for authorisation of health claims (EFSA NDA Panel, 2011).

As outlined in the EFSA general guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016), it is the responsibility of the applicant to provide the totality of the available evidence.

2.2. Methodologies

The general approach of the NDA Panel for the evaluation of health claim applications is outlined in the EFSA general guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016).

The scientific requirements for health claims related to functions of the nervous system, including psychological functions, are outlined in a specific EFSA guidance (EFSA NDA Panel, 2012).

3. Assessment

3.1. Characterisation of the food/constituent

The food constituent that is proposed by the applicant as the subject of the health claim is DHA (22:6 n-3).

In the original application, the food was specified as 'DHA, alone or in combination with EPA'. Following an EFSA request for clarification, the applicant indicated that DHA is the food which is the subject of the health claim.

DHA is a well-characterised n-3 long-chain polyunsaturated fatty acid (LCPUFA) that can be quantified in foods by established methods. The absorption of DHA is well documented. This evaluation applies to all sources of DHA in the specified amounts.

The Panel considers that the food constituent, DHA, which is the subject of the health claim, is sufficiently characterised.

3.2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant is 'contributes to improved memory function'. The target population proposed by the applicant is 'the general healthy adult population'.

Memory is the cognitive ability to maintain previously learned information, so that it may be accessed and used at a later time. Memory is not a unitary construct but instead reflects a number of distinct cognitive processes (e.g. episodic memory, working memory, short-term memory, semantic memory).

The improvement, maintenance or reduced loss of one or more cognitive processes related to memory is considered to be a beneficial physiological effect (EFSA NDA Panel, 2012).

Changes in different aspects of memory (e.g. episodic memory, working memory, short-term memory, semantic memory) can be measured *in vivo* in humans using valid psychometric tests.

The Panel considers that an improvement of memory function is a beneficial physiological effect.

3.3. Scientific substantiation of the claimed effect

The applicant performed a literature search in Ovid/Medline and Embase through January 2013. Relevant terms representing DHA and EPA (as well as their dietary sources) and memory were used. Hand searching was carried out by examining the reference lists of all relevant studies, pertinent review articles and meta-analyses. The literature searches were repeated in September 2014 to identify any recently published trials. Studies were included if they were controlled clinical trials (randomised and non-randomised) or observational studies and assessed the effect of DHA intake, either alone or in combination with EPA, from conventional or fortified sources (foods or food supplements), on memory outcomes in healthy adults residing in the community at baseline (includes assisted living facilities, but excludes long-term care nursing facilities), with or without mild memory complaints. Studies were excluded if: (a) intakes of DHA and/or EPA were not reported; (b) they were carried out in subjects with current diagnosis of Alzheimer's disease (AD), dementia, vascular dementia, stroke, head injury, substance abuse, metabolic disturbance, depression, behavioural or neurologic disorder; (c) included subjects with group mean baseline mini-mental state examination (MMSE) scores < 24 (indicative of advanced cognitive decline/AD); (d) more than 10% of the subjects were on psychotropic, antidepressant, stimulant or drugs approved for the treatment of AD/dementia.

The applicant identified 15 human intervention studies and 13 observational studies as being pertinent to the claim. The applicant also provided three meta-analyses using data from the 15 intervention studies.

3.3.1. Human intervention studies

One of the human intervention studies provided (Richter et al., 2010) was a single-arm trial (i.e. no control group) on the effects of phosphatidylserine (PS) with DHA and EPA on episodic and working memory. The Panel considers that no conclusions can be drawn from this uncontrolled study for the scientific substantiation of the claim.

Johnson et al. (2008) conducted a double-blind, randomised controlled trial (RCT) on the effects of DHA (from microalgae) and/or lutein consumed for 4 months on episodic memory, working memory, short-term memory and semantic memory in 57 women aged between 60 and 80 years. Within-group differences between memory scores at baseline and after supplementation were tested with paired *t*-tests. Between-group comparisons were not reported. The applicant was requested to provide a statistical analysis of the data which is appropriate for placebo-controlled designs, i.e. an analysis which tests differences in changes among the study arms over time for the memory outcomes measured. Such an analysis was not provided by the applicant. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

Kotani et al. (2006) studied the effects of DHA in combination with arachidonic acid (ARA) on episodic memory, semantic memory and short-term memory in patients with mild cognitive impairment (MCI), organic brain lesions and AD. A total of 21 patients (12 male, 9 female, mean age = 61.8 years) with MCI were randomly allocated to receive daily 240 mg DHA in combination with 240 mg ARA (both contained in a food supplement) or 240 mg placebo. A group of 10 patients with organic brain lesions and eight patients with AD also received the DHA+ARA supplement, but were not randomised to the placebo. The duration of the study was 90 days. The statistical analyses consisted of within-group comparisons of changes from baseline to the end of the study. Between-group comparisons were not reported. As for the previous study (Johnson et al., 2008), the applicant was requested to provide a statistical analysis of the data which is appropriate for placebo-controlled designs, but no such analysis was provided by the applicant. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

The remaining 12 human intervention studies are discussed below. The studies were performed with DHA derived from microalgae (n = 2), fish oil (n = 9) and with PS-DHA (n = 1).

3.3.1.1. Human intervention studies performed with DHA derived from microalgae

Yurko-Mauro et al. (2010) conducted a double-blind, parallel, multi-centre RCT on the effects of DHA on episodic and working memory in healthy older adults with age-related cognitive decline (ARCD). The Diagnostics and Statistical Manual (DSM-IV) defines ARCD as an 'objectively identified decline in cognitive functioning consequent to the aging process that is within limits given a person's age. Individuals may report problems remembering names or appointments or may experience

difficulty solving complex problems' (American Psychiatric Association, 1994). ARCD was identified as a baseline immediate or delayed recall score ≥ 1 SD below the mean of adults aged 25–35 years on the Logical Memory subtest of the Wechsler Memory Scale (WMS Version III, 1997). A total of 485 participants aged ≥ 55 years with ARCD (282 female, 203 male) were recruited at 19 study sites. Participants were excluded if they had a MMSE score < 26, or a mean daily consumption of > 200 mg DHA in the 2 months before randomisation. Participants were randomised to consume 900 mg of either DHA (single cell oil from *Schizochytrium* sp.) or placebo (50% corn oil/50% soy oil) daily for 24 weeks. All supplement capsules were orange flavoured, and identical in size and appearance. The mean age for both groups was 70 years. Episodic memory was assessed by the Cambridge Neuropsychological Test Automated Battery (CANTAB) Paired Associate Learning Test (PAL), the CANTAB Pattern Recognition Memory (PRM) test and the CANTAB Verbal Recognition Memory (VRM) test (Robbins et al., 1994). Working memory was assessed by the CANTAB Spatial Working Memory (SWM) test. Assessments were undertaken at baseline, 12 and 24 weeks. A power calculation for PAL 6 pattern error scores indicated that a total of 325 participants were needed to detect an effect size of 0.19, with an α-level of 0.05 and a power of 80%, considering a 10% dropout rate. CANTAB scores at 24 weeks were analysed by ANCOVA with factors of treatment (DHA; placebo), site, age group (55–69 years; ≥ 70 years) and education. Baseline scores were included as covariate. A total of 437 participants completed the study. Results of the intention-to-treat (ITT) analysis (with the last observation carried forward for missing data) showed that the DHA group had significantly fewer PAL 6 pattern errors at week 24 compared to the placebo group ($p = 0.032$). The DHA group also had more VRM correct responses for both immediate recall ($p = 0.018$) and delayed recall ($p = 0.012$). There were no significant treatment group differences for either PRM performance or SWM performance. The Panel notes that this study shows a positive effect of consuming 900 mg of DHA per day for 24 weeks on episodic memory, but not on working memory, in older adults with ARCD.

Benton et al. (2013) conducted a double-blind, parallel RCT on the effects of DHA on episodic and semantic memory in 305 females (mean age 21.8 years), who were assigned to consume either 400 mg of DHA (derived from *Crypthecodinium cohnii*) or placebo (corn oil) per day for 50 days. The study was conducted in two phases, with one half of the participants taking part 3 months after the first half. Episodic memory was assessed by a recall of word list test in which three matched lists of 30 nouns were created for the study. The test involved presentation of a list at the rate of one word per second, with recall measured by participants writing down as many of the words they could remember in a 2-min period. Measures of both immediate and delayed (25 min) recall were obtained at baseline, 25 days and 50 days. Semantic memory was measured by a recall of capitals test in which participants were asked to name the capital cities of 30 countries. When a capital could not be recalled, participants rated their 'feeling of knowing' on a 6-point scale. The test was administered twice at the 50-day assessment, but only to half of the participants (i.e. those taking part in the second phase of the study). No power calculation was reported. A total of 285 participants completed the trial and were included in the analyses. The Panel notes that data analyses were provided for the population of completers only. Results for episodic memory were analysed by three-way repeated-measures (RM)-ANOVA with factors of treatment (DHA; placebo), time (baseline, day 25, day 50) and recall time (immediate; delayed). Results are reported as means ± SEM. There was a significant three-way interaction for recall of word lists ($F(2,566) = 4.41$, $p < 0.01$), with more words forgotten by the DHA group (3.2 ± 0.19) than by the placebo group (2.6 ± 0.19, $p < 0.01$) at the end of the study. There were no significant between-group differences for the semantic memory recall of capitals test. The Panel notes that this study shows a negative effect of consuming 400 mg/day of DHA for 50 days on episodic memory and no effect on semantic memory in young adults.

3.3.1.2. Human intervention studies performed with DHA from fish oil

Karr et al. (2012) conducted a double-blind, parallel RCT on the effects of DHA and EPA on episodic memory in 43 college students (29 female), who received either 2,000 mg fish oil (480 mg DHA, 720 mg EPA) or 2,000 mg placebo (coconut oil) per day for 4 weeks. Episodic memory was assessed with the Rey Auditory Verbal Learning Test (RAVLT) (Lezak, 1995) which was administered at baseline and again after 4 weeks. The test involved completion of seven stages in which participants recalled words from a list of 15 (List A) which were read out to them. Stages 1–5 involved five presentations of List A, each followed by immediate recall. This was followed by an interference trial involving presentations and recall of a second list (List B). Stage 6 entailed recall of the original List A after the interference effects of List B, and in stage 7 participants recalled List A after a 20-min delay. Performance was measured by the number of correctly recalled words on stages 1–7, and by two

summary measures calculated by subtracting the number of words correct on stage 5 from the number correct on stages 6 and 7. No power calculations were reported. Results for episodic memory were analysed by RM-ANOVA with factors of treatment (fish oil; placebo), time (baseline, week 4) and varying numbers of RAVLT stages (stages 1–5, stage 6, stage 7, summary measure 7-5 and summary measure 6-5). Two participants, one from each study group, were removed from the analyses due to 'problems with following supplementation procedures'. The Panel notes that data analyses were provided for the population of completers (n = 41) only. There were no significant differences between groups for stages 1–5. Treatment × time interactions for stage 6 ($F(1,39) = 4.45$, $p = 0.04$) and stage 7 ($F(1,39) = 5.65$, $p = 0.02$) were both statistically significant. A significant treatment × time interaction was also found for the summary measure 7-5 ($F(1,39) = 4.30$, $p = 0.045$). *Post hoc* comparisons to assess at which time point the intervention and control groups differed regarding the RAVLT scores were not performed. The applicant was therefore requested to provide *post hoc* analyses of the significant treatment × time interactions which would establish whether the fish oil group achieved significantly better memory performance than the placebo group at the end of the period of supplementation. In reply, the applicant referred to the significant treatment × time interactions as reported in the publication but did not provide any further analyses. The Panel notes that, for each significant time × treatment interaction, performance of the fish oil group improved across the duration of the study, while performance of the control group declined (RAVLT scores (mean ± SD) for stages 6 and 7 at baseline: placebo = 11.57 ± 2.36 and 11.48 ± 2.96, respectively, and fish oil = 9.85 ± 3.28 and 10.00 ± 3.43, respectively; at week 4: placebo = 11.33 ± 2.06 and 10.29 ± 3.26, respectively, and fish oil = 10.80 ± 3.14 and 10.50 ± 3.61, respectively). However, the Panel also notes that the apparent differences in the RAVLT scores between the fish oil and the placebo groups at baseline, rather than a real treatment effect, may have driven the time × treatment interactions. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

Sinn et al. (2012) performed a double-blind, parallel RCT on the effects of DHA-rich and EPA-rich fish oils on episodic memory, short-term memory, working memory and semantic memory in 50 volunteers aged > 65 years (16 female) with MCI, defined as signs of cognitive decline beyond those expected for age, but not dementia. Participants were randomly allocated to receive daily either EPA-rich fish oil (0.16 g DHA, 1.67 g EPA; n = 17), DHA-rich fish oil (1.55 g DHA, 0.40 g EPA; n = 18) or placebo (safflower oil providing 2.2 g linolenic acid; n = 15) for 6 months. The mean ages of the three randomised groups ranged from 73 to 74.9 years. Episodic memory was assessed by the RAVLT. Short-term memory was assessed with the Digits Forward subtest of the Wechsler Adult Intelligence Scale (WAIS) (Wechsler, 1997), and working memory was assessed with the Digits Backward subtest of the WAIS. Semantic memory was assessed by the Boston Naming Task (Lezak, 1995) and verbal fluency test (Bryan et al., 1997) in which participants produced as many words as possible in 1 min either beginning with a designated letter (Initial Letter Fluency) or not containing a designated letter (excluded letter frequency). Memory was assessed at baseline and again at the end of the study. In the publication, it is reported that, 'according to Cohen's *f*, the required *n* to detect a medium effect size ($d = 0.50$) in a RM within–between interaction design for three groups with a power of 0.80 is 66 participants (22 per group)'. From this description, the Panel is unclear as to which variable was used for power calculations and how these calculations were performed. A total of 40 subjects completed the study (control group n = 11; DHA-group n = 16; EPA-group n = 13). Linear mixed models were used to analyse the effects of DHA-rich or EPA-rich fish oil vs. placebo in the ITT population (n = 50). The application of a linear multilevel model was considered appropriate for the pattern of missing values. All information could be used and predicted values could be obtained for all participants at each time point. Only Initial Letter Fluency scores (semantic memory) significantly improved in the DHA-rich fish oil group compared with the placebo group ($p = 0.04$). The EPA-rich fish oil and placebo groups did not differ significantly. There were no significant effects involving any of the other memory measures. The Panel notes that no correction of the significance level was made to take into account multiple comparisons. The Panel considers that this study with a daily consumption of 1.55 g DHA from fish oil for 6 months did not show an effect on episodic, short-term, working or semantic memory in subjects with MCI.

Jackson et al. (2012) conducted a double-blind RCT on the effects of DHA-rich and EPA-rich fish oils on episodic memory, working memory and semantic memory in 159 volunteers. Participants were randomly allocated to receive a daily intake of EPA-rich fish oil (200 mg DHA, 300 mg EPA), DHA-rich fish oil (450 mg DHA, 90 mg EPA) or placebo (olive oil) for 12 weeks. Mean ages of the three randomised groups ranged from 21.9 to 22.7 years. Episodic memory was assessed by tests of Immediate Word Recall, Delayed Word Recall, Delayed Word Recognition, Delayed Picture Recognition

and Names-to-Faces Recall. Working memory was assessed by tests of Numeric Working Memory, Alphabetic Working Memory, SWM (Corsi Blocks), Three-back Task and Telephone Number Working Memory. Semantic memory was assessed by a test of verbal fluency. All tests were taken from the Computerised Mental Performance Assessment System (url: http://www.cognitivetesting.co.uk/). Memory was assessed at baseline and again at the end of the study. Data were analysed by one-way between-subject (treatment group) ANCOVA, with data from the relevant outcome measure at baseline as covariate. A priori planned comparisons using *t*-tests were made between placebo and both the DHA-rich fish oil and EPA-rich fish oil groups. A total of 140 subjects were compliant with the study protocol and entered data analyses (control group = 48, DHA-group = 46, EPA-group = 46). The Panel notes that data analyses were provided for the per protocol (PP) population only. There was a treatment effect for Names-to-Faces Recall ($F(2,136) = 3.73$, $p = 0.026$), with participants in both the DHA-rich fish oil group and EPA-rich fish oil group showing fewer correct matches than the placebo group ($p = 0.047$ and 0.013, respectively). There were no significant effects for any of the remaining memory tests. The Panel notes that no adjustment to the significance level was made to take into account the multiple comparisons. The Panel considers that this study with a daily consumption of 450 mg DHA from fish oil for 12 weeks did not show an effect on episodic, working or semantic memory in young adults.

Dangour et al. (2010) completed a double-blind, parallel RCT on the effects of DHA on episodic memory, working memory and semantic memory in 867 cognitively healthy adults aged between 70 and 79 years (390 female). Participants were randomly allocated to receive a daily intake of fish oil (500 mg DHA, 200 mg EPA) or placebo (olive oil) for 24 months. Episodic memory was assessed by the California Verbal Learning Test (CVLT; including a test of memory of a 16-item word list) (Delis et al., 1987), immediate and delayed recall of a short story taken from the WMS (Wechsler, 1987), a test of spatial memory (van Niekerk et al., 2004) and three tests of prospective memory (Maylor, 1993). Working memory was assessed with the Digits Backward subtest of the WAIS (Wechsler, 1981), and semantic memory was assessed by a verbal fluency test (Goodglass and Kaplan, 1983). Short-term memory was assessed with the Digits Forward subtest of the WAIS. Memory tests were administered twice, at baseline and again at the end of the study. A composite episodic memory score was calculated by summing *z*-scores for the CVLT, location memory and story recall measures. All other memory measures were included in a global cognitive function composite score which included several other non-memory measures such as processing speed, reaction time and executive function. Therefore, the Panel considers that no conclusions can be drawn from this global cognitive function composite score for the scientific substantiation of the claim. A power calculation indicated that a total of 332 participants were required per group to detect a 0.3 SD difference in delayed free recall of a 16-item word list between treatment groups. Data were analysed by one-way between-subject (treatment group) ANCOVA, with data from the baseline as the covariate, as well as age, sex and age at leaving full-time education. A total of 748 participants completed the study, with cognitive function data available for 744 participants (fish oil group = 375, control group = 369). The Panel notes that data analyses were provided for the population of completers only. There were no significant differences between treatment groups for the total words correct in the first three CVLT trials, or the number of words recalled in CVLT delayed recall. There were no significant differences between groups in the composite memory scores at 24 months. The Panel notes that the daily consumption of 500 mg DHA from fish oil for 24 months did not have an effect on episodic, short-term, working or semantic memory in cognitively healthy older adults.

Lee et al. (2013) conducted a double-blind RCT on the effects of fish oil supplementation on episodic memory, working memory and short-term memory in participants with MCI. A total of 36 elderly participants with MCI (27 female) were randomly allocated to receive a daily dose of DHA-rich fish oil (1.29 g DHA, 450 mg EPA) or placebo (corn oil, providing 1.8 g linolenic acid) for 12 months. Episodic memory was assessed by the RAVLT, and two subtests from the Wechsler Memory Scale-Revised (WMS-R) (Wechsler, 1984). Working memory was assessed with the Digit Span Backward subtest of the WAIS (Wechsler, 1997), and short-term memory was assessed with the Digit Span Forward subtest of the WAIS. Memory was assessed at baseline, 6 months and 12 months. No power calculation was reported. Changes in memory scores for individual tests were assessed using RM-ANCOVA, with factors of treatment (fish oil; placebo) and time (baseline; 12 months) and covariates of baseline age, baseline systolic and diastolic blood pressure, and years of schooling. Bonferroni corrections were used for multiple comparisons in *post hoc* analyses of all neuropsychological tests. There were significant treatment × time interactions for digit span ($F = 9.89$; $p < 0.0001$) and RAVLT delayed recall ($F = 3.99$; $p < 0.05$). Absolute data were provided as means

and 95% CI (digit span, placebo, baseline: 8.5 (7.554–9.529), 6 months: 8.4 (7.242–9.472), 12 months: 8.0 (6.877–9.113); fish oil group, baseline: 8.0 (6.994–9.036), 6 months: 8.2 (7.058–9.363), 12 months: 9.6 (8.437–10.749)); RAVLT delayed recall, placebo, baseline: 6.1 (4.431–7.860), 6 months: 6.4 (4.687–8.052), 12 months: 5.0 (3.587–6.312); fish oil group, baseline: 6.7 (4.897–8.442), 6 months: 6.1 (4.399–7.878), 12 months: 8.1 (6.645–9.462). There were no significant effects involving any of the other memory tests. A composite memory score was calculated by the authors by averaging *z*-scores for the RAVLT, WMS-R and Digit Span Backward tests. Changes in memory performance from baseline to 12 months were calculated for this composite memory score, and the change was significantly better for the fish oil group than the placebo group (difference between groups in composite *z*-score: 0.799, 95% CI: 0.339–1.258, $p < 0.01$). The Panel considers that this study showed an improvement of memory function with a daily consumption of 1.29 g DHA from fish oil for 12 months in adults with MCI.

Stonehouse et al. (2013) reported the findings of a double-blind, parallel RCT of the effects of DHA supplementation on episodic and working memory. A total of 228 healthy adults (145 female) aged between 18 and 45 years and with a 'low' dietary intake of DHA (i.e. less than about 200 mg EPA+DHA/week) were randomly allocated to receive fish oil (1.16 g DHA, 0.17 g EPA) or placebo (high oleic acid sunflower oil) daily for 6 months. Episodic memory was assessed by tests of Immediate Word Recall, Delayed Word Recall, Delayed Word Recognition and Delayed Picture Recognition. Working memory was assessed by tests of Letter-Number Sequencing, SWM (Corsi Blocks) and n-back Tasks. All tests were taken from the Computerised Mental Performance Assessment System. Memory was assessed at baseline and again at the end of the study. Composite memory scores were calculated by obtaining the average of the *z*-scores for individual memory tests. The composite scores included memory, working memory, reaction time of memory and reaction time of working memory. Power calculations indicated that a total of 32 participants per sex and treatment group were required to detect a difference in *z*-score of 0.5 'for memory domains' at a power of 0.8 at a significance level of 0.05. Changes in memory during the treatment period between fish oil and placebo groups were assessed by using ANCOVA models to adjust for baseline cognitive function test scores and other covariates, as follows: education, first language (English compared with other), age and baseline blood DHA concentrations. Sex and presence of the apolipoprotein E4 (APOE4) allelic variant were added as independent variables to test for sex × treatment, APOE × treatment, and APOE × sex × treatment interactions. A total of 176 participants ($n = 85$ in the DHA group (52 women); $n = 91$ in the placebo group (58 women)) completed the trial and were included in the analyses. Dropouts did not differ significantly from the participants who completed the study for any baseline characteristic except age. The Panel notes that data analyses were provided for the population of completers only. The sex × treatment interaction for episodic memory was significant ($p = 0.01$), with women in the DHA group showing a greater improvement compared to placebo ($p = 0.01$), but no significant differences between groups were observed for men ($p = 0.20$). There was a significant treatment effect for reaction time of episodic memory, with the DHA group responding faster than placebo, but the sex × treatment effect was not significant. There was a similar effect of treatment for reaction time of working memory with the DHA group responding faster than placebo ($p = 0.002$). The sex × treatment interaction was also significant ($p = 0.03$) with men in the DHA group completing the task faster than men in the placebo group ($p = 0.001$), but no significant difference was reported for women ($p = 0.39$). There were no significant effects for working memory. APOE4 status did not affect treatment responses for any memory outcome. The Panel notes that in this study with a consumption of 1.16 g/day DHA from fish oil for 6 months, a number of improvements were observed on episodic and working memory in healthy adults, but these effects were inconsistent among tests and sexes. The Panel considers that this study does not show consistent effects of DHA consumption on memory.

Stough et al. (2012) completed a triple-blind, parallel RCT of DHA in tuna oil on episodic and working memory. A total of 112 healthy adults aged 45–80 years were randomly assigned to receive daily DHA-rich fish oil (252 mg DHA, 60 mg EPA) or placebo (soybean oil) for 90 days. The Panel notes that no demographic data were provided for the ITT population ($n = 112$), but only for the PP population ($n = 74$). Episodic memory was assessed by tests of Immediate Word Recall, Delayed Word Recall, Delayed Word Recognition and Delayed Picture Recognition. Working memory was assessed by tests of SWM and Numeric Working Memory. All tests were taken from the cognitive drug research (CDR) computerised assessment system (Wesnes et al., 1999). The individual measures were used to calculate CDR factor scores for Speed of Memory, Secondary Memory and Working Memory. Memory tests were administered at baseline and again at the end of the study. *Post hoc* power calculations indicated that, with a sample size of 74 participants and an α-level of 0.05, there was a 99% chance of detecting a 'medium' effect size ($f = 0.25$) but only a 40% chance of detecting a 'small'

effect size (f = 0.1) 'on cognition'. The Panel notes that the outcome variable was not further specified. Nineteen participants withdrew during the course of the trial. A further 19 participants were removed because of inadequate compliance (i.e. < 90%). The remaining 74 participants (fish oil = 38; placebo = 36) were included in the final analyses. The Panel notes that data analyses were provided for the PP population only. Mixed-design RM-ANCOVA, with time (baseline vs. post-treatment) as the within-subjects factor and treatment group (DHA vs. placebo) as the between-subjects factor, and age as a covariate, were conducted on all cognitive outcomes. There were no significant effects for any of the memory measures. The Panel notes that the consumption of 252 mg/day DHA in fish oil for 90 days did not have an effect on episodic or working memory in healthy adults.

The double-blind, three-arm, parallel RCT of Van de Rest et al. (2008) investigated the effect of fish oil supplementation for 26 weeks on measures of episodic memory, working memory, semantic memory and short-term memory. A total of 302 cognitively healthy participants (135 female) with a mean age of 70 years and MMSE scores > 21 were randomised to consume daily a 'high dose' of fish oil providing 847 ± 23 mg DHA and 1,093 ± 17 mg EPA, a 'low dose' of fish oil providing 176 ± 4 mg DHA and 226 ± 3 mg EPA, or placebo (mainly oleic acid). Episodic memory was assessed by the Word Learning Test (Van der Elst et al., 2005), working memory with the Digits Backward subtest of the WAIS (Wechsler, 1981), short-term memory with the Digits Forward subtest of the WAIS and semantic memory with the Verbal Fluency Test (Van der Elst et al., 2005). A composite memory score was calculated as the mean of the z-scores for Immediate and Delayed Word Learning, Word Learning Recognition and Backward Digit Span. Assessments were undertaken three times (at baseline and after 13 and 26 weeks). A power calculation based on the Word Learning Test required a minimum of 63 participants per group to detect a difference of 4 points with a power of 80% and an α-level of 0.05. RM-ANOVA was used to test differential changes among the three intervention groups after 13 and 26 weeks, with the treatment group as the factor and scores on the memory composite and short-term memory test as the dependent variables. A Dunnett's *post hoc* test was conducted to compare mean changes in the two treatment groups with changes in the control group. There were no significant effects of treatment at any time point. The Panel notes that the consumption of 850 mg/day DHA in fish oil for 26 weeks did not have an effect on episodic, working, short-term or semantic memory in cognitively healthy older adults.

The double-blind, parallel RCT of Witte et al. (2013) examined the effect of DHA and EPA supplementation on measures of episodic memory, working memory, semantic memory and short-term memory. A total of 65 healthy participants (30 female) aged 50–75 years were randomised to receive either fish oil (880 mg DHA, 1,320 mg EPA) or placebo (sunflower oil) daily for 26 weeks. The mean ages of the two groups were not significantly different (DHA+EPA = 65 years; placebo = 62.9 years), and all participants had a MMSE score > 26. Episodic memory was assessed by the Auditory Verbal Learning Test (AVLT) (Lezak, 2004), which included measures of immediate and delayed recall, as well as recognition. Working memory was assessed with the Backward Digit Span Test, and short-term memory with the Forward Digit Span Test (Lezak, 2004). Semantic memory was assessed with the Verbal Fluency Test (Lezak, 2004). A composite memory score was calculated by averaging z-scores for AVLT learning, delayed recall, recognition and digit span backward. No power calculations were reported. Results were analysed with a RM-ANOVA with factors of treatment (fish oil; placebo) and time (baseline; week 26), and Bonferroni corrections for multiple comparisons. There were no significant effects for the composite memory score, and no significant effect of treatment on short-term memory. The Panel notes that the consumption of 880 mg/day DHA in fish oil for 26 weeks did not have an effect on episodic, working, semantic or short-term memory in healthy adults.

3.3.1.3. Human intervention study performed with PS-DHA

Vakhapova et al. (2010) examined the effects of PS-DHA on measures of episodic memory, working memory and short-term memory in 157 (78 female) non-demented elderly participants with memory complaints. Inclusion criteria were complaints of memory loss in everyday life as confirmed by a score of ≥ 25 on the Memory Complaint Questionnaire scale (Crook et al., 1992); absence of dementia as determined by a MMSE score ≥ 27 for participants with college education and ≥ 26 for all others, and a Clinical Dementia Rating Scale score ≤ 0.5; and scores in NexAde computerised cognitive assessment tool ≤ the mean norm (Aharonson and Korczyn, 2004). Participants were randomly assigned to receive PS-DHA (providing 300 mg PS and 79 mg DHA+EPA (DHA/EPA ratio of 3:1)) or placebo (cellulose) daily for 15 weeks. The mean age of both groups was 73 years. Episodic memory was assessed by the RAVLT, Rey Complex Figure Test (RCFT) (Meyers and Meyers, 1995), and immediate and delayed pattern recall tasks from the NexAde battery. Working memory was assessed with a digit span

backward task, and short-term memory with a digit span forward task, both from the NexAde battery. NexAde memory composite scores were calculated from the individual tests: memory recognition and recall (episodic memory), and spatial short-term memory. Assessments were undertaken twice, at baseline and at the end of the study. No power calculation was reported. Changes were examined with ANCOVA, which included baseline performance and MMSE scores as covariates. A total of 131 subjects completed the study (n = 66 in the PS-DHA group; n = 65 in the placebo group). Nine further subjects (six from the placebo group and three from the PS-DHA group) were excluded from the analyses for lack of compliance (i.e. < 65%). The Panel notes that data analyses were provided for the PP population (n = 122) only, with the exception of the immediate recall scores, which were provided for the PP population and the completers (n = 131). At 15 weeks, immediate recall scores in the RAVLT were significantly higher ($p = 0.041$) in the PS-DHA group compared to the placebo group in the PP population but not in the population of completers. There were no significant effects on any other RAVLT outcomes (i.e. total learning, delayed recall and recognition) or the RCFT. No adjustment to the significance level was made to take into account the multiple comparisons. No statistically significant differences between the groups were observed for any of the NexAde tasks, including tests for working memory and short-term memory. The Panel notes that, in this study, only one out of four RAVLT outcomes was significantly higher in the PS-DHA than in the placebo group (in the PP population only), that no correction for multiple comparisons was applied, and that there were no statistically significant differences for working memory or short-term memory. The Panel considers that, overall, this study with a consumption of DHA taken as PS-DHA for 15 weeks did not show an improvement of memory function.

The Panel notes that out of the 11 intervention studies from which conclusions can be drawn for the scientific substantiation of the claim, two were performed with DHA from microalgae, eight with DHA from fish oil and one with PS-DHA. One study conducted with DHA from algal oil showed an improvement in episodic memory but not in working memory in healthy older adults with ARCD after consumption of 900 mg DHA for 24 weeks (Yurko-Mauro et al., 2010), while the other study with DHA from the same source showed a negative effect on episodic memory and no effect on semantic memory in young adults after consumption of 400 mg DHA for 50 days (Benton et al., 2013). Out of the eight intervention studies performed with DHA from fish oil, which provided daily doses of DHA ranging from 252 mg to 1.55 g for periods between 12 weeks and 24 months, only one showed an improvement in memory in adults with MCI (Lee et al., 2013), while the results from another study were inconsistent (Stonehouse et al., 2013) and six studies did not show an effect of DHA supplementation on memory outcomes (Van de Rest et al., 2008; Dangour et al., 2010; Jackson et al., 2012; Sinn et al., 2012; Stough et al., 2012; Witte et al., 2013). The study which used PS-DHA did not show an effect of the food on memory function (Vakhapova et al., 2010). The Panel considers that the majority of the human intervention studies provided did not show an effect of DHA supplementation on memory, and that the conflicting results across studies cannot be explained by differences in the study design, the source of DHA, the DHA dose, the baseline characteristics of the subjects recruited, the sample size or the duration of the studies.

3.3.2. Meta-analyses as provided by the applicant

The applicant conducted meta-analyses using data from the 15 intervention studies described in the previous section. These meta-analyses (partly published by Yurko-Mauro et al., 2015) summarised 62 data points for episodic memory, 15 data points for semantic memory and 21 data points for working memory. Both between-group (differences from placebo) and within-group (differences from baseline) comparisons were provided for episodic, semantic and working memory. Random effects meta-analyses were conducted to generate weighted group mean differences and Hedge's *g* scores, which standardised the weighted group mean differences by the pooled standard deviation of the studies. The studies included in the meta-analyses were conducted in healthy subjects without (n = 9 studies) and with (n = 6 studies) mild memory complaints, ranged from 28 to 730 days in duration (average 4–6 months), were mostly (n = 11 studies) conducted with older adults (45–80 years of age), provided up to 1.55 g DHA/day and had a sample size of 8–867 subjects.

The overall between-group models, which included all dose levels of DHA, all sources of DHA and all subjects regardless of their age or cognitive status at baseline, showed no effect of DHA supplementation on episodic, semantic or working memory.

Sensitivity analyses considering age, baseline cognitive status and DHA dose levels were also provided. However, the Panel considers that the results from those analyses were inconsistent and provided no clear evidence for an effect of DHA on memory function.

The Panel considers that these meta-analyses do not provide evidence for an effect of DHA on memory function.

3.3.3. Observational studies

The applicant provided 13 observational studies as pertinent to the claim (Kalmijn et al., 2004; Whalley et al., 2004; Beydoun et al., 2007; de Groot et al., 2007; Dullemeijer et al., 2007; Whalley et al., 2008; Muldoon et al., 2010; Kesse-Guyot et al., 2011; Milte et al., 2011; Samieri et al., 2011; Phillips et al., 2012; Tan et al., 2012; Titova et al., 2013).

Among the references provided, nine studies (Beydoun et al., 2007; de Groot et al., 2007; Dullemeijer et al., 2007; Whalley et al., 2008; Muldoon et al., 2010; Milte et al., 2011; Samieri et al., 2011; Phillips et al., 2012; Tan et al., 2012) did not report on dietary intakes of DHA but addressed the association between blood concentrations of DHA and measures of memory. One study (Titova et al., 2013) reported only a composite cognitive score but no independent memory test scores. The Panel considers that no conclusions can be drawn from these studies for the scientific substantiation of the claim.

Three studies (Kalmijn et al., 2004; Whalley et al., 2004; Kesse-Guyot et al., 2011) addressed the association between dietary intakes of DHA or fish oil supplements and measures of memory. Kalmijn et al. (2004) completed a cross-sectional study with 1,613 participants aged between 45 and 70 years and found no significant association between DHA+EPA intake and risk of episodic memory impairment. Whalley et al. (2004) completed a cross-sectional study with 301 participants aged 64 years and found no significant difference in episodic memory scores between fish oil users and fish oil nonusers. Kesse-Guyot et al. (2011) completed a prospective cohort study with 3,294 adults aged 35–60 years and found no significant association between DHA intake and episodic memory scores. The Panel notes that, in these studies, the consumption of DHA showed no association with episodic memory in adults.

The Panel considers that the three observational studies from which conclusions can be drawn for the scientific substantiation of the claim do not provide evidence for an association between dietary DHA and memory function.

3.3.4. Weighing of the evidence

In weighing the evidence, the Panel took into account that out of the 11 human intervention studies from which conclusions can be drawn for the scientific substantiation of the claim, two studies showed a beneficial effect of DHA supplementation on memory function, one study showed inconsistent results, one study showed a negative effect of DHA on memory function and seven studies did not show an effect of DHA on memory outcomes. The Panel considers that the majority of the human intervention studies provided did not show an effect of DHA supplementation on memory, and that the conflicting results across studies cannot be explained by differences in the study design, the source of DHA, the DHA dose, the baseline characteristics of the subjects recruited or the duration of the studies. The Panel also took into account that the meta-analyses of the intervention studies submitted by the applicant do not provide evidence for an effect of DHA on memory function, and that the three observational studies from which conclusions could be drawn for the scientific substantiation of the claim do not provide evidence for an association between dietary DHA and memory function.

The Panel concludes that a cause and effect relationship has not been established between the consumption of DHA and an improvement of memory function.

4. Conclusions

On the basis of the data presented, the Panel concludes that:

- The food constituent, DHA, which is the subject of the health claim, is sufficiently characterised.
- The claimed effect proposed by the applicant is 'contributes to improved memory function'. The target population proposed by the applicant is 'the general healthy adult population'. An improvement of memory function is a beneficial physiological effect.
- A cause and effect relationship has not been established between the consumption of DHA and an improvement of memory function.

Steps taken by EFSA

1) Health claim application on DHA, alone or in combination with EPA, and contributes to improved memory function pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0438_UK). Submitted by DSM Nutritional Products, 6450 Dobbin Road, Columbia, MD 21405, USA.
2) The application was received by EFSA on 24 July 2015.
3) The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.
4) The scientific evaluation procedure started on 15 September 2015.
5) On 30 October 2015, the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was suspended on 11 November 2015, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
6) On 20 November 2015, EFSA received the applicant's reply and the scientific evaluation was restarted, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
7) On 20 January 2016, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was suspended on 5 February 2016, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
8) On 18 February 2016, EFSA received the applicant's reply and the scientific evaluation was restarted, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
9) On 30 March 2016, the NDA Panel, having evaluated the data submitted, adopted by written procedure an opinion on the scientific substantiation of a health claim related to DHA and improved memory function.

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Abbreviations

AD	Alzheimer's disease
APOE4	apolipoprotein E4
ARA	arachidonic acid
ARCD	age-related cognitive decline
AVLT	Auditory Verbal Learning Test
CANTAB	Cambridge Neuropsychological Test Automated Battery
CDR	cognitive drug research
CI	confidence interval
CVLT	California Verbal Learning Test
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
ITT	intention-to-treat
MCI	mild cognitive impairment
MMSE	mini-mental state examination
PAL	Paired Associate Learning
PP	per protocol
PRM	Pattern Recognition Memory
PS	phosphatidylserine
RAVLT	Rey Auditory Verbal Learning Test

RCFT	Rey Complex Figure Test
RCT	randomised controlled trial
RM	repeated-measures
SD	standard deviation
SE	standard error of the mean
SWM	Spatial Working Memory
VRM	Verbal Recognition Memory
WAIS	Wechsler Adult Intelligence Scale
WMS	Wechsler Memory Scale
WMS-R	Wechsler Memory Scale-Revised